

Calcium Cyclotron Resonance and Diatom Mobility

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The hypothesis that movement of biological ions may be predicted by cyclotron resonance theory applied to cell membranes is tested in these experiments. Diatoms (*Amphora coffeaeformis*) were chosen as the biosystem since they move or don't move, depending on how much calcium is transported across the membrane. The experiments demonstrate that a particular ion (calcium) is apparently moved across the cell membrane in response to the DC and AC values of magnetic flux densities (B) and the frequency derived from the cyclotron resonance theory. A clear resonance is shown and a rather sharp frequency response curve is demonstrated. The experiments also show a dose response as the AC value of the flux density is varied, and that odd harmonics of the basic cyclotron frequency are also effective.

Key words: ELF fields, cell mobility, calcium ion effects

INTRODUCTION

It has been suggested in a number of recent papers [McLeod and Liboff, 1986; Liboff, 1985] that the influence on the life processes of biological cells by externally applied, low-level magnetic fields can be explained through the application of cyclotron resonance theory. The theory, as applied to cells, is suggested by the fact that the cyclotron resonance frequencies for several biologically important ions such as calcium, sodium, potassium, magnesium, etc, all fall in the 10-100-Hz range when the static magnetic field, per the theory, is assumed to be in the amplitude range of the earth's field. There is a very limited amount of experimental evidence that cyclotron resonance in cells may exist [Liboff, personal communication; Thomas et al, 1986] although this evidence has been interpreted in alternate ways [Blackman et al, 1985]. One thing made clear in the Blackman et al [1985] experiments, however, is that static magnetic field in the vicinity of the experiment does play a role in how low-energy applied fields interact with a biosystem.

The work reported in this paper is an attempt to demonstrate with a simple cell that a single ion can be selected and its movement across a membrane controlled with the proper combination of DC and AC magnetic flux densities (MFD). The frequency

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of the AC MFD and the magnitude of the DC MFD are set by the use of the cyclotron resonance theory developed in previous papers [McLeod and Liboff, 1986; Allis et al, 1963; Liboff and McLeod, 1986]. The biological system was chosen to be one well studied (by biologists concerned with surface fouling) and also one that has a clear ion-membrane related process which can be studied either in or out of the applied magnetic field. *Amphora coffeaeformis* (diatom) is known to be mobile or not mobile depending on the amount of Ca^{++} that is available outside of the cell membrane [Cooksey, 1981; Cooksey and Cooksey, 1980]. Movement of this ion across the membrane controls the ability of the diatom to move. The results of these experiments clearly demonstrate, for the first time, that resonance is achieved at the predicted frequency, the process has a well-defined frequency response curve, and it is probable that the process is taking place through the cell membrane. We also demonstrate that certain harmonics of the cyclotron resonance frequency are effective in controlling the calcium movement. By increasing the amplitude of the applied AC field past the optimum point, the experimental data seem to indicate an excess of calcium moved across the membrane and resulted in less mobility again.

MATERIALS AND METHODS

Diatoms

Amphora coffeaeformis, marine diatoms, were inoculated into glass 15-ml culture tubes containing 5 ml of sterile pH 7.6 ASP-2 medium [Cooksey, 1981] with 0.25 mM Ca^{++} . This medium provides for rapid growth without adhesion of the diatoms to the glass [Cooksey, 1981]. The tubes were then placed in an incubator at 25 °C and exposed to 5,400-lux continuous illumination from daylight-type fluorescent lamps. After 5 days of culture, the tubes were removed and shaken on a vortex stirrer. The cells were then centrifuged at 20 °C for 20 min at 3,000 g. The supernatant was discarded, and the cells were resuspended and recentrifuged twice in 10 ml of a minimal medium (M-M) composed of buffered salts containing 12 μM Ca^{++} as a contaminant [Cooksey, 1981]. After the final spin, the cells were resuspended in 5 ml of M-M and counted in a standard hemocytometer. They were then diluted to 1.5×10^5 cells/ml and brushed onto agar plates with a sterile No. 3 red sable watercolor brush. This procedure resulted in the diatoms resting on the surface of the agar, since the liquid brushed on with the cells was absorbed by the agar gel.

Plates

Plates were made by dissolving 2 g of Difco plain powdered agar in 100 ml of M-M, to which was added 0.0, 0.25, 0.5, or 5.0 mM of Ca^{++} as CaCl_2 . The agar was then pressure sterilized and poured into sterile 60-mm plastic culture dishes. After setting, the plates were stored for at least 2 days under refrigeration at 4 °C to allow for diffusion of CO_2 back into the agar. Without this step, the diatoms were not mobile. Plates prepared in this manner were streaked with diatoms, removed to the experimental apparatus, and placed as closely as possible to the center of the coils' air spaces.

Apparatus

The test apparatus is pictured in Figure 1, and consisted of three pairs of coils placed in a Helmholtz configuration. One pair of bucking coils, with their magnetic

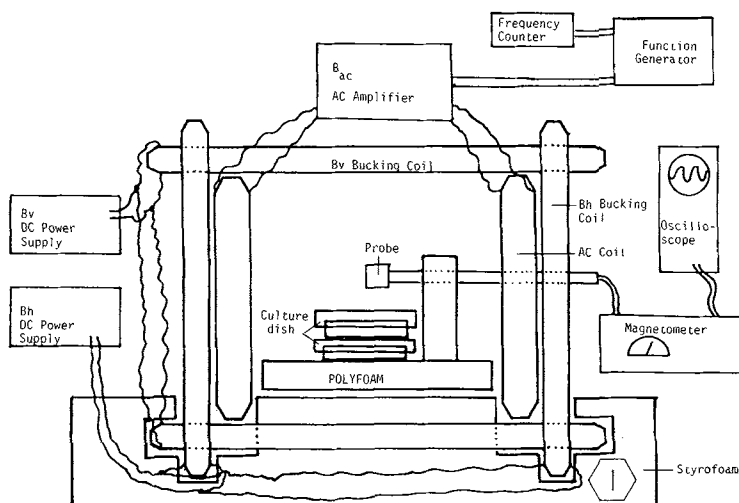


Fig. 1. Experimental apparatus. Labels are self-explanatory. Consult text for details of coil windings and electronics.

axis along the z-axis, controlled the vertical component of the static MFD (B_v). These coils consisted of 250 turns of B & S 22-gauge enameled copper magnet wire, and were 23 cm in diameter. They were energized with a Hewlett Packard 6206 B DC power supply.

A second set of 23-cm-diameter bucking coils, consisting of 500 turns of B & S 24-gauge enameled copper magnet wire, were placed with their axis along the x-axis (north-south) to control the horizontal component of the static MFD (B_H). These coils were energized by a Sorensen QRB 30-1 DC power supply. No coils were used to control the y-axis, (east-west), since it could be brought to 0 by orienting the x-axis directly along the north-south axis of the earth's magnetic field.

A third set of coils, 20 cm in diameter and composed of 960 turns of B & S 24-gauge enameled copper magnet wire were placed just inside the x-axis coils, to superimpose an AC flux density (B_{AC}) on the static B_H .

The AC coils were driven with a sine wave produced by a Feedback TWG-500 variable-phase function generator fed through a Hewlett-Packard/Harrison 6824A power supply/amplifier. The frequency of the ac field was checked with a Hewlett-Packard 5324A universal counter, and both frequency and amplitude were observed on a Tektronix 561A oscilloscope. The static MFD strengths were observed directly from the meter of a Dowty/RFL Industries, Inc., SAM-21 single-axis magnetometer (range: 30–100 μ T), while the peak alternating MFD was observed via the oscilloscope fed from the magnetometer's analogue amplifier output. The scope display was calibrated using a standard Keithley 169 digital voltmeter (DVM). The magnetometer was calibrated using a standard set of coils whose properties had been previously determined using instruments referable to the National Bureau of Standards.

Mobility vs Ca^{++} Concentration

In order to verify the experiments of others which had demonstrated the dependence of mobility on external Ca^{++} concentration, we established a series of plates containing 0.012, 0.05, 0.1, 0.25, 0.50, and 5.0 mM Ca^{++} . Cells were

streaked onto these plates, then left for 1 h, either in a Ca^{++} resonance field (16 Hz, 20.9 μT ; see below) or outside the field.

Magnetic Flux Densities (MFD)

The MFD's employed were those calculated from the resonance theory model [McLeod and Liboff, 1986]. For most experiments, B_v was set to 0.0. Assuming an AC frequency of 16 Hz, which avoided the resonance points for Na^+ , K^+ , and Mg^{++} , the Ca^{++} resonant static MFD was calculated to be 20.9 μT (.209 G).

To provide evidence of a resonance effect from these trials, we established a series of plates, in which we varied the Ca^{++} concentration and the *frequency of the AC field*. For these tests, B_H was set at 20.9 μT , $B_v=0$, and $B_{AC}=20.9 \mu\text{T}$. The frequencies used were 16, 32, 48, and 64 Hz, establishing a fundamental plus three harmonics series. The Ca^{++} concentrations varied as follows: 5.0, 0.5, 0.25, 0.0 mM.

To assess the effect of an alternating flux in the absence of a static MFD or vice versa, B_H and B_v were brought to 0, while the alternating flux was applied at 16, 32, 48, and 64 Hz at 20.9 μT peak. This experiment was done only on plates containing 0.25 mM Ca^{++} , since the effects of the first experiment were most dramatic at that concentration. To assess the effect of B_H and B_v in the absence of an alternating field, 0.25 mM Ca^{++} plates were exposed to static fields where $B_v=0$, and $B_H=20.9, 41.8, 62.7, \text{ and } 83.6 \mu\text{T}$.

In order to be consistent with the cyclotron resonance model, one of the tests which may be done is to orient the magnetic fields at right angles to one another. If the effects are truly the result of ion cyclotron resonance, the AC and DC fields must be parallel [McLeod and Liboff, 1986]. Accordingly, we established plates at 0.25 mM Ca^{++} , then set the horizontal static MFD to 0 and the vertical static field at 20.9 μT . The AC flux was then established horizontally at 20.9 μT peak and 16 Hz.

To test for the possibility that the AC amplitude applied to the diatoms might be critical, we established cultures on 5 mM and 0.25 mM Ca^{++} plates, and then subjected them to the following conditions: $B_H=20.9 \mu\text{T}$; $B_v=0$; $B_{ac}=5.2, 7.0, 10.5, 20.9, 41.8, \text{ and } 62.7 \mu\text{T}$. This series afforded us a range of AC values which should have demonstrated a magnetic flux density dose-reponse effect, if any was present.

To ascertain whether it was indeed Ca^{++} which was the ion responsible for the effect, we established plates containing 0.25 mM and 5 mM Ca^{++} , then added LaCl_3 , a potent competitive inhibitor of Ca^{++} attachment to Ca^{++} receptor sites. The La^{+++} concentration range was set at $10^{-3}, 10^{-4}, 10^{-5}, \text{ and } 10^{-6}$ M. In other experiments [Mayer et al, 1972] these concentrations had proven sufficient to span the range from complete inhibition to complete lack of effectiveness. Half of the plates served as controls, receiving no exposure to the magnetic fields, while the other half received a maximum effective stimulus ($B_H=20.9 \mu\text{T}$; $B_v=0$; $B_{AC}=20.9 \mu\text{T}$; 16 Hz).

Finally, to ascertain how sharply the resonance is defined, we ran plates containing 0.25 mM Ca^{++} at 12, 14, 16, 18, 20, and 32 Hz with $B_H=20.9 \mu\text{T}$, $B_v=0$, and $B_{AC}=20.9 \mu\text{T}$. This series should have provided a maximum resonance point at 16 Hz, with submaximal response on either side of 16 Hz.

Controls

Controls for each experiment consisted of a series of plates established in identical fashion to the experimental plates. These were then placed on the same table as the experimental apparatus, but kept at least 50 cm away from the coils. At this distance, the alternating field was undetectable as measured on the SAM-21 (limit of resolution = $.03 \mu\text{T}$), and the measured change in the normal static fields was never more than $0.1 \mu\text{T}$. The controls were, of course, exposed to the normal earth's magnetic field. In our Lab, B_H was $16 \mu\text{T}$ and B_V was $52 \mu\text{T}$, with very minor daily fluctuations in strength and x-axis orientation. The ambient 60-Hz field averaged $0.1 \mu\text{T}$ peak.

General Conditions

The experiments were carried out at least in triplicate in a laboratory at a temperature of $20^\circ\text{C} \pm 1^\circ\text{C}$. At the table surface, light intensity was 500 lux. The support table consisted of a sheet of wooden composition board supported on concrete blocks. All electronic equipment was kept at least 1.50 m from the test table to eliminate vibration and stray fields, and to keep equipment heat transfer to negligible levels. No ferrous materials were allowed within 1.50 m of the test table. The coils were supported by closed-cell Styrofoam blocks, while the test plates were supported by open-cell polyurethane foam blocks; this arrangement limited heat transfer from the coils to the plates (see Fig. 1). The small number of plates used was occasioned by two factors: the restricted area of uniform field within the coils, and the relatively small variability in the experiments, which led to early statistical significance.

Data Collection and Analysis

Test and control plates were allowed to run for 1 h under the various experimental conditions. Then the plates were removed from the apparatus and immediately examined under a normal light microscope. All scoring was done on a blind basis. Diatoms which were mobile left clearly visible tracks on the agar plates. The cells were scored as either mobile or nonmobile, depending upon the presence or absence of a visible track extending beyond the edge of the frustule ($> 10 \mu\text{m}$). Based on accepted practice when scoring diatom mobility, we did not attempt to quantify track length or orientation with respect to magnetic field lines. Only single, nonclumped cells were counted, and counting was done on the basis of contiguous fields along the streaked line of cells. A minimum of 100 diatoms were scored on each plate, and the percentage of mobile cells per plate was calculated.

Standard deviations of the mean percent mobilities were calculated from the results of the runs for each experiment, and all error bars in the figures and tables are standard deviations ($N = \text{No. of plates}$). Since the data were nonparametric and not complex, they were also analyzed by χ^2 . Some t-statistics were performed on mean percentages, but the results indicated that χ^2 was more discriminative for these experiments, and so t-tests were abandoned. All P-values in tables, text, or figures are based on χ^2 . Comparison (ANOVA) of the controls from all experiments showed that they could be legitimately pooled where useful. Since the diatoms we used are a clone, we have selected the single diatom as the independent unit of measure, and have assumed that diatoms on the same plate, or on different plates receiving the same treatment, will respond similarly. Another experimental unit may not yield exactly the same significance levels. It is our belief, based on sample t-statistical

calculations where differences between groups were small, that the use of χ^2 statistics (upper tail probabilities, n by n) revealed the same information as would have been revealed by another test.

RESULTS

Mobility vs Ca^{++} Concentration

The results of these experiments may be seen in Figure 2. They confirm the previous observations of Cooksey [1981]. Without the presence of the field, the cells cannot move at Ca^{++} concentration below 0.25 mM. Mobility reaches about half maximum at 0.5 mM. In the resonance field, mobility is barely evident at 0.05 mM, but quite prominent at 0.10 mM. At 0.25 mM, mobility in the field is approximately maximal. If one compares these data to those of Cooksey [1981], the correlation is quite accurate for the nonstimulated cells. He found that the cells reach maximum mobility at 2.5 mM. If one uses that figure for saturation, it suggests that the application of the correct MFD moves about ten times as much Ca^{++} into the cell as would normally occur.

Varying Frequency and Ca^{++} Concentration

For this trial, it is evident from Figure 3 that the controls again followed the same pattern as for the previous group. There was a minor effect of the applied magnetic fields at 5 mM Ca^{++} concentration. The results at 32 and 64 Hz were significantly higher than controls ($P < .05$) using a t-test, but not with χ^2 . At 16 and 48 Hz, there was no significant difference. At 0 mM Ca^{++} , the results at 16 Hz were significant ($P < .05$) with a t-test, but not with χ^2 . However, at 0.5 mM Ca^{++} the mobility was approximately doubled when B_H was 20.9 μT , and the alternating MFD was 20.9 μT , peak at 16 or 48 Hz. There was a minor field effect at 32 Hz ($P < .05$), and none at 64 Hz. At 0.25 mM Ca^{++} , the results were again quite dramatic. As in the first experiment, 16 Hz at 20.9 μT B_H produced about a seven-fold increase in

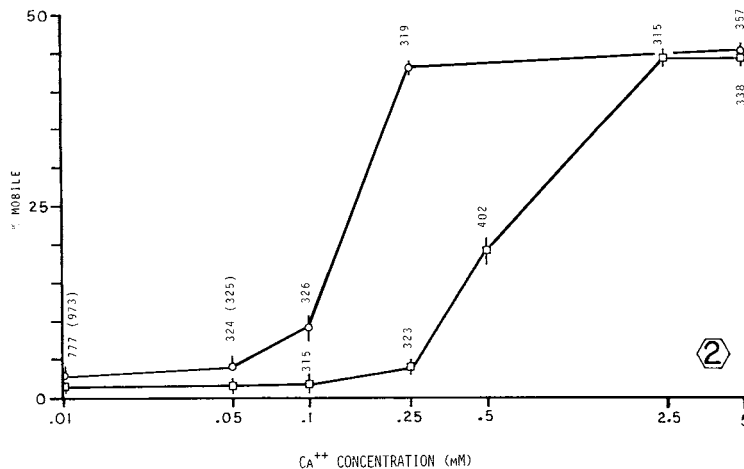


Fig. 2. Ca^{++} Concentration vs mobility. Open squares = controls (no field); open circles = plates tuned for Ca^{++} resonance (16 Hz), $B_H = 20.9 \mu\text{T}$; $B_{AC} = 20.9 \mu\text{T}$. Error bars are $\pm$ SD (3 plates for each point except for .01 mM, where the number for controls is 7, and that for field-exposed is 9). P-values were calculated by χ^2 from cell counts. The numbers by the data points are the cell counts. The field-exposed cells were significantly different from controls ($P \leq .01$) only at 0.1, 0.25, and 0.5 mM.

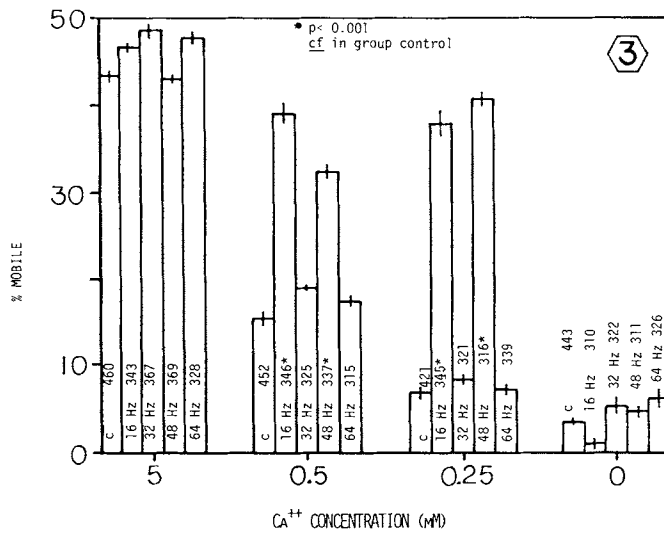


Fig. 3. Ca^{++} concentration vs mobility vs AC frequency ($B_H = 20.9 \mu\text{T}$; $B_{AC} = 20.9 \mu\text{T}$). Error bars are $\pm$ SD (3 plates for each point except controls, where the number was 4). N's on the figure represent No. of cells counted, and the asterisks identify highly significant ($P < .001$) differences between the controls for each group and data points within that group, as calculated by χ^2 .

TABLE 1. No AC Field: $B_v = 0$, B_H Varies, $\text{Ca}^{++} = 0.25 \text{ mM}$ (SD Is Based on No. of Plates)

Condition	N (plates)	No. of cells	% Mobility $\pm$ SD
Control	12	1,398	4.9 ± 2.2
20.9	3	341	2.9 ± 0.8
41.8	3	347	6.6 ± 0.8
62.7	3	315	4.8 ± 3.4
83.6	3	315	4.8 ± 3.4

mobility. This was repeated when the frequency was raised to 48 Hz. The data were significant when compared to controls. There were no significant differences between controls and experimentals at 32 and 64 Hz.

Effect of AC Field

When the AC field was eliminated, the static field had no statistically significant effect on mobility (Table 1).

Effect of DC Field

When the DC field was eliminated and the AC field was varied, the results were essentially identical with those described above. The AC field had no effect if the static field was not present (Table 2).

Effect of Perpendicular Fields

When the static and alternating magnetic flux densities were perpendicular to one another, they were totally ineffective. Plates treated in this fashion show no elevation of mobility over that of control plates which had not been exposed to fields. Table 3 illustrates the results.

TABLE 2. No DC Field: $B_B=0$, $B_V=0$, f Varies, $Ca^{++}=0.25$ mM, $B_{AC}=20.9$ μ T (SD Based on No. of Plates)

Condition (Hz)	N (plates)	No. of cells	% Mobility $\pm$ SD
Control	12	1,365	6.3 ± 0.9
16	3	323	4.3 ± 1.2
32	3	375	6.2 ± 1.3
48	3	340	5.0 ± 0.5
64	3	370	5.4 ± 0.9

TABLE 3. Perpendicular Fields: $B_H=0$, $B_V=20.9$ μ T, $B_{AC}=20.9$ μ T, $f=16$ Hz, $Ca^{++}=0.25$ mM (SD Based on No. of Plates)

Condition	N (plates)	N (cells)	% Mobility $\pm$ SD
Control (see Table 1)	12	1,398	4.9 ± 2.2^a
Fields on	3	336	5.7 ± 1.3

^aControls for this series were the same as for Table 1.

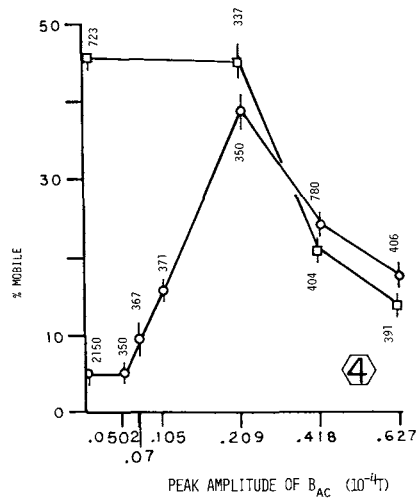


Fig. 4. Mobility vs peak AC amplitude at Ca^{++} resonance. ($B_H=20.9$ μ T; 16 Hz). Open squares = 5.0 mM plates; open circles = 0.25 mM plates. Error bars are $\pm$ SD; N=3 plates except for control at 0 and 4.8 μ T (20 and 7 plates), and field on at 0 μ T (7 plates). N's on the figure represent No. of cells counted, and P's were calculated by χ^2 . From zero field to 20.9 μ T, the curves are significantly different ($P < .01$). Thereafter they are not different.

Effect of AC Amplitude

The results of the tests using a variety of AC amplitudes are graphically illustrated by Figure 4. At 5.02 μ T the fields were ineffective. Above this value, however, increasing the AC magnetic flux density produced nearly linear increases in diatom mobility on the 0.25 mM plates, until the B_{AC} reached 20.9 μ T peak. When B_{AC} rose to 41.8 and 62.7 μ T, mobility decreased on both the 0.25 mM and the 5 mM plates and was progressively inhibited, dropping to approximately three times the level seen with control 0.25 mM plates. Mobility at this field level was approximately 30% of maximum.

Effects of LaCl_3

The results of the tests using lanthanum chloride may be seen in Figure 5. The La^{+++} ion clearly inhibited mobility at all concentrations above 1×10^{-6} M when the Ca^{++} concentration was 0.25 mM. At the higher Ca^{++} concentration of 5 mM, inhibition was evident at all concentrations, but was much less at 1×10^{-6} than at higher concentrations. When the magnetic fields were applied, inhibition was at least partially reversed for all concentrations of La^{+++} when the Ca^{++} concentration was 5 mM. The reversal was small at $\text{La}^{+++} = 1 \times 10^{-3}$ M, but was statistically significant ($P < .05$). Mobility never returned to 100% of control, but was clearly evident at La^{+++} concentrations of 1×10^{-4} M and lower. When the Ca^{++} concentration was lower, at 0.25 mM, the reversal of inhibition seemed more like a normal dose response. No reversal occurred at 1×10^{-3} M La^{+++} , and then a gradual reversal occurred as La^{+++} concentration decreased. At 1×10^{-6} M La^{+++} , the field-exposed dishes containing 0.25 mM and 5 mM Ca^{++} were not statistically different.

Resonance Curve

The tests to establish the sharpness of the resonance suggested why careful control of the MFD's and the frequency was essential. As may be seen from the curve in Figure 6, the resonance was quite distinct. The normal maximum for the 0.25 mM Ca^{++} plates was reached at 16 Hz (38% mobility), while the mobilities at 12 and 20 Hz were nearly symmetrically depressed, being 17.9 and 18.8%, respectively. At 14 and 18 Hz, the values were 22.7% and 23.9%. These data were used with a computer graphics curve fitting technique to estimate the damping factor τ discussed in the

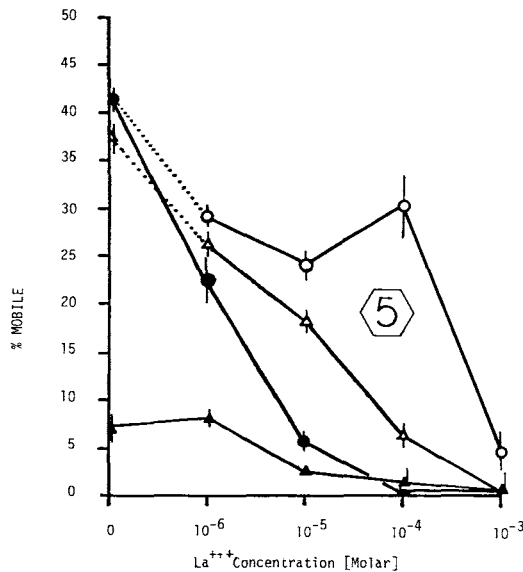


Fig. 5. Mobility vs La^{+++} concentrations with or without a field tuned for Ca^{++} . ($B_H = 20.9 \mu\text{T}$; $B_{AC} = 20.9 \mu\text{T}$; 16 Hz). Closed triangles = 0.25 mM Ca^{++} , no field; closed circles = 5.0 mM Ca^{++} , no field; open triangles = 0.25 mM Ca^{++} , field on; open circles = 5.0 mM Ca^{++} , field on. Error Bars are $\pm$ SD; $N = 3$ plates. No. of cells counted omitted for clarity on the figure (mean No. = 382; range = 322–461). See text for statistical comparisons.

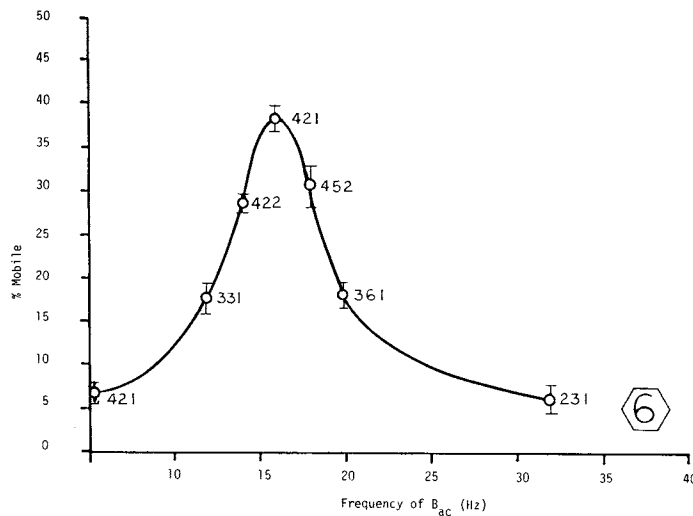


Fig. 6. Resonance curve: Variable AC frequency. $B_H = 20.9 \mu\text{T}$; $B_{AC} = 20.9$. Error Bars are $\pm$ SD; $N = 3$ plates at 12 and 20 Hz; 4 plates at 0, 14, 16, and 18 Hz; and 2 plates at 32 Hz.

theory [Allis et al, 1963; Liboff and McLeod, 1986]. For these data, τ is estimated to be 0.047 s.

DISCUSSION

The results of this series of experiments support the ion cyclotron resonance model as an important factor in the biological effects of applied time-varying magnetic fields. The experiments varying frequency while holding the magnitude of B_{AC} and B_H constant gave results that are obviously consistent with the resonance model, and further support it since the resonance follows a $2n+1$ pattern (ie, the resonance occurring at values of $1n, 3n, 5n \dots$ etc). Note in Figure 3 that 16 Hz through 48 Hz give data to support this statement. The experiments omitting either B_H or B_{AC} make it clear that both are necessary, and this is further confirmed by the experiment applying the fields orthogonally. If the resonance is indeed cyclotronic, no effect of orthogonal fields should be seen [LcLeod and Liboff, 1986]; nor was there any. The frequency series reveals that the resonance is quite distinct. The curve shape suggests from theoretical considerations that the effective collision time for the Ca^{++} ion in the channel where the effect may be occurring is about 47 ms [Liboff and McLeod, 1986]. The fact of $2n+1$ resonance rather than some other harmonic arrangement suggests that the boundary conditions of the ion channel are important, and that the resonance is not occurring at the membrane surface. The channel energy function as suggested by Liboff and McLeod [1986] has a high energy level at each end and a low energy level in the channel. While this energy picture is speculative, it suggests that boundary conditions are placed on the energy of an ion transversing the membrane at either end of the channel and perhaps also at the channel midpoint. If, for instance, the energy function cannot have a minimum at midchannel, then only 1, 3, 5, etc, wave functions will resonate in this "cavity", since even numbers of wave functions will all have a zero at midchannel. The $(2n+1)$ resonance pattern seen in

the data of this experiment lend some credence to the postulated energy pattern. These data may suggest a quantized set of energy states within the channel.

It is important to emphasize that this model suggests the ion does not transverse the membrane in a simple straight path, nor is the ion viewed as a free particle in a plasma. Thus, a simplistic calculation for the collision time, or the transit time of an ion across the membrane will yield results considerably different from those suggested by the model and this experimental work. The 47-ms collision time obtained by fitting the theoretical conductivity resonance curve to the experimental data is indeed surprisingly long. As we have noted elsewhere (unpublished data), however, this number is consistent with collision times obtained by fitting (in the same fashion) data taken by others on other systems and in other labs [Bawin and Adey, 1976; Dutta et al, 1984]. The reader is invited to refer to the last sentence of the previous paragraph again. If the channel has a set of quantum energy states which make it more complex than a simple "hole," it is possible that free space values of wavelength, collision time, transit time, etc, do not apply.

Further papers, now in preparation, will explore the limits of the system with respect to harmonics and frequency response.

The response of the biosystem to changes in the AC MFD indicate that the theoretical model must contain an AC component. Durney (personal communication) calculated results that indicated a system, at cyclotron resonance, could be significantly perturbed with an AC component added to the normal static magnetic flux density. The argument that the energy input supplied by the AC MFD is too low for the system to utilize has been debated for some time. The data reported here, however, clearly show that the diatom does react to an AC MFD that ranges from about 5 to 63 μT . The dose-response of Figure 4 strongly suggests this aspect of the effect. When the system is driven with B_{AC} greater than the optimum value, it appears as though too much Ca^{++} enters the cells, and inhibits mobility. This inhibitory effect of too much Ca^{++} is well known for these organisms [Cooksey and Cooksey, 1980; Cooksey and Chansang, 1976], so the suggestion is perhaps not too far afield. Below optimum values, not enough Ca^{++} crosses the membrane to obtain complete mobility.

The data in Figure 4 showing a change in mobility when the amplitude of the AC MFD changes raises a question about the exact role played by the AC flux density. Note that since B_H and B_{AC} are applied in parallel (recall B_{AC} swings about the tip of the B_H vector), and since the magnitude of B_{AC} is on the order of or greater than the magnitude of B_H , the vector magnitude of the total MFD is far from constant. B_H is always present of course, but the sum of the two applied fields varies in time. The experimental data in Figure 4 seem to indicate the diatoms do not react to the sum of the MFD's, but to each flux density separately. The conditions for cyclotron resonance are set by B_H and the frequency of B_{AC} . The maximum reached as the magnitude of B_{AC} is varied suggests an AC coupling factor that is an addition to the cyclotron resonance theory.

It is also too early to exclude other factors from the AC amplitude response. One could certainly propose situations where AC amplitude could effect the availability of ions or their absorption to receptor sites on the membrane and their subsequent entrance into the channels. Finally, the biological effect seen could certainly be a function of the rate at which or efficiency with which Ca^{++} ions can be processed and utilized by the cell's biochemical machinery. Our current research is directed toward exploring these questions, since the answers are expected to shed considerable light on the mechanism of controlling specific ion movement.

The experiments with lanthanum give support to the statement that Ca^{++} ions at the membrane are responsible for the effect. Aside from the fact that the tuning of the resonance was specifically adapted to Ca^{++} , La^{+++} is known to be, among other things, a strong competitive inhibitor of Ca^{++} attachment sites on the cell membrane [Mayer et al, 1972]. Our present concept to explain the results is as follows: At high La^{+++} concentrations (1 mM), all Ca^{++} sites on the membrane are effectively blocked, and essentially all Ca^{++} transport and movement are also blocked. As the concentration of La^{+++} is lowered, progressively more transport channels are available, though the total Ca^{++} transport is still too low to allow for movement of the diatoms. However, when the fields are applied, the increased Ca^{++} transport through the remaining "open" channels is enough to raise the internal Ca^{++} concentration in the cells above the threshold for movement. The apparent dose response as La^{+++} concentration is lowered is a function of the number of available Ca^{++} channels and the applied field.

Alternatively, if one wished to argue that it is some other ion which is responsible for the effect, it is hard to imagine how the experimental results would account for that. Sodium, potassium, and magnesium concentrations were the same in all experiments, yet the effect clearly was dependent upon Ca^{++} ion concentration, as was mobility in the absence of any fields. One could perhaps argue that Ca^{++} is merely a cofactor which must be present for mobility, but the net result is the same. Frequency values consistent with a model for calcium ion involvement in ion cyclotron resonance did significantly alter mobility in these experiments, whatever the details of the channel mechanism may be. Those details are of considerable importance, of course, but must be addressed by other experiments. Our experiments give little hint as to what those mechanisms may be, but the data may suggest that a new view of the membrane channel is necessary.

It should be pointed out that since only about 45% of the cells ever move in these experiments, it would be possible to argue that only those cells oriented in a particular fashion within the field are affected. That possibility can be eliminated because of the anatomy of the diatom. The reason only 45% of the cells move is quite simple: these organisms are constructed a bit like a turtle. That is, they have a definite "top" and "bottom." The membranes responsible for movement only project through the "bottom" of the siliceous frustule. When one paints them onto the surface of the agar, they have an even chance of landing on the agar with the top or bottom up. Only those which land top up can move. The others are confined to lying still with their "feet" waving in the air. They cannot roll over. Thus, a statistical maximum of 50% would be expected to be mobile. Allowing for a few dead, damaged, or otherwise incapacitated cells, mobility of 45% represents very nearly complete mobility under the circumstances of these experiments. Such would certainly not be the case if some particular orientation of the organisms were required. Examination of the plates reveals that the painting on process does not orient the organisms. They are randomly oriented. Neither do they orient to the static field. The tracks they leave in the agar go off in every conceivable direction, even though we purposely aligned the strip of painted-on cells parallel with the static field. If there were any orienting effect of the fields, it would seem unlikely that the tracks would wander about randomly.

In sum, we believe that we have rather convincingly demonstrated that the effects seen with time-varying magnetic fields in these experiments are the result of ion cyclotron resonance. This then, may explain the often bewildering claims and

counter claims in the literature regarding the effect of AC magnetic fields on biological systems. If no account is taken of the static magnetic field, or of the orientation of the coils used in a particular experiment, it is not inconceivable that an experiment may work at one location and not in another location [Liboff, 1985; Thomas et al, 1986; Blackman et al, 1985]. Even if the orientation of the coils were carefully controlled, fairly small variations in B_H and/or B_V from place to place and from time to time could move the fields into and out of resonance, thus making results variable, even within the same laboratory.

The fact of magnetic field effects is abundantly clear. There is far too much evidence from too many separate laboratories to convincingly argue otherwise. We hope that these experiments may help to lay a solid foundation for the understanding of the mechanistic aspects of the observed effects, and may lead to more uniformly predictable experimental designs and results.

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